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Review article

How are Growth and Luminescence Regulated Independently In Light Organ Symbionts?

J. WOODLAND HASTINGS[1], JOHN MAKEMSON[2] and PAUL V. DUNLAP[3]

[1] *Department of Cellular and Developmental Biology*
Harvard University, Cambridge, MA 02138, U.S.A.

[2] *Department of Biological Sciences*
Florida International University, Miami, FL 33199, U.S.A.

[3] *Department of Biology*
New Mexico State University, Las Cruces, NM 88003, U.S.A.

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Abstract

A key feature in symbiotic mutualisms is the establishment of some sort of equilibrium between host and symbiont, especially with regard to their relative growth rates. Although the imposition of host restriction of symbiont growth would seem simple enough, the contribution(s) provided to the host by the symbiont should presumably *not* be restricted. Symbioses involving light organs in teleost fishes model the problem elegantly: pure cultures of luminous bacteria are maintained in the organ at a high cell density where growth rate is slow, but luminescence is continuous and intense. How is it possible to constrain growth so severely yet maintain maximal light emission?

Studies of growth and luminescence as related to culture conditions have been made for 4 different species of luminous bacteria, 3 of which occur in light organs of different fish species. Nutrient limitation may restrict both growth and the development of luminescence, but the latter may be subject to a specific stimulation. In media with low oxygen or low iron, cells grow slowly, but in some species may be intensely luminescent, with a high luciferase content and light emission per cell. Differential effects on growth and luminescence are also related to the osmolarity of the medium in some species. Host regulation of these (and possibly other) factors could explain the physiological control of growth and luminescence of symbionts, but no information is available concerning whether or not, and if so how, these postulated factors are controlled

within light organs. Since all light organs communicate with the exterior, the infection by the symbiont strain may be postulated to occur from the outside by way of that channel. Since factors responsible for physiological control of growth and luminescence in light organ may be idiosyncratic to a particular species or strain, host control of such factors can in principle contribute to the development and maintenance of a pure culture.

Keywords: bioluminescence, luciferase, light organs, bacteria, teleosts, growth, symbiont regulation

1. Introduction

A number of marine fishes (and also squids) are bioluminescent by virtue of luminous bacteria that are cultured as light organ symbionts in specially structured and sometimes highly adapted light organs (Herring and Morin, 1978; Hastings and Nealson, 1981). In these mutualisms the bacteria are provided with "room and board" while the fish acquires the ability to emit light. The existence of specialized organs and their coevolution with specific symbionts is known in numerous diverse types of symbioses in invertebrates (Buchner, 1965). But the luminescent associations appear to be unique in that a vertebrate maintains a pure culture of a prokaryote in specialized organ. Many species from several families of teleost fish possess such light organs, which differ with regard to location, openings to the exterior, structural components, and the species of bacteria cultured (Fig. 1; Table 1).

Actually, such bacteria are not limited to this habitat; the same species also occur in sea water as planktonic forms, and appear to possess an impressive ecological versatility (Nealson and Hastings, 1979). Presumably, they can move back and forth from the symbiont to free living state, enjoying two "life styles". In some cases, it should be emphasized, the bacterial symbiont has not been cultured and/or identified, so knowledge of their physiology is lacking. It should also be mentioned that *Vibrio harveyi*, a major planktonic species in surface waters, used extensively in studies concerned with the regulation of luminescence, is not known to occur as a light organ symbiont. However, it does form associations with higher organisms, for example as a gut symbiont, and is therefore useful to consider in modeling mechanisms in symbiosis.

We will discuss here 3 specific but interrelated aspects of fish light organ symbioses: how the hosts might be able to control the relative rates of growth and luminescence of the symbionts, how the bacteria are acquired, and how a pure culture is maintained.

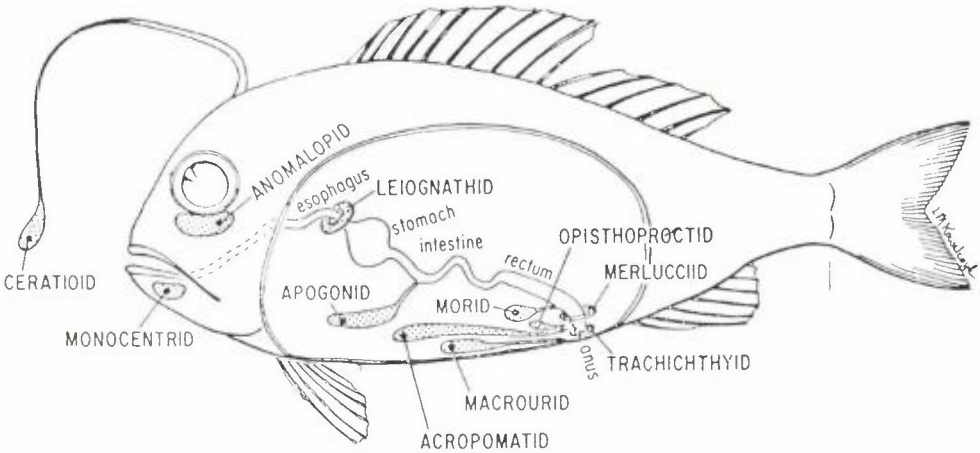

Figure 1. "Ichthylicht", a diagrammatic fish used here to indicate the approximate locations, sizes, and openings of light organs of the various groups of luminescent fishes (see Table 1 for details).

Table 1. Luminous bacteria cultured in specialized teleost light organs

Bacterial species	Host fish families	Fish habitat
Vibrio fischeri[a]	Monocentridae	Shallow—100 m, temperate
Photobacterium leiognathi[b]	Leiognathidae	Shallow, tropical
	Apogonidae	Shallow, tropical
	Acropomatidae[c]	Deep, cold (?)
Photobacterium phosphoreum	Macrouridae	Deep cold
	Merlucciidae	Deep, cold
	Opisthoproctidae	Deep, cold
	Trachichthyidae	Deep, cold
	Moridae	Deep, cold
	Chlorophthalmidae[d]	Deep, cold
Bacterial symbionts not cultured	Anomalopidae	Shallow
	Ceratioidei (9 families)	Deep

[a] Also occur in light organs of sepiolid squids (G. Leisman and K.H. Nealson, unpublished; P.V. Dunlap and S. Fukasawa, unpublished).

[b] Also occur in light organs of loliginid squids (Fukasawa and Dunlap, 1986)

[c] Tentative identification (P.V. Dunlap and S. Fukasawa, unpublished)

[d] P.V. Dunlap, G. Sarantinos and S. Fukasawa, unpublished)

2. Maximum Luminescence with Minimum Growth

At an experimental level the first question can be modeled to some extent in pure cultures. In mutualisms, a key feature is the establishment of an equilibrium between host and symbiont with regard to their relative growth rates. For reasons of energy expenditures, successful bacterial symbionts are those that are somehow restrained from doing what bacteria normally do best: active and rapid growth. Superficially, this may seem to present no particular problem: one has only to postulate a growth restriction by the host on the procaryotic symbiont. But the situation is probably more complicated. Since a symbiont presumably provides some specific benefit, it behooves the host to maximize that benefit. Thus growth and the beneficial system must presumably be regulated independently.

This problem is beautifully modeled (Nealson, 1979) by the fish light-organ symbiosis: the luminous bacteria are maintained in pure culture (Reichelt et al., 1977; Fitzgerald, 1977) at a high cell density in specialized organs, where they are provided with a niche and nutrients, while the ability to emit light is conferred upon the fish. The bacteria grow within the organ at a slow rate compared to growth in a rich medium (Haygood et al., 1984; Nealson et al., 1984; Dunlap, 1984b), but their luminescence is intense, as high or higher per cell than is generally achieveable in cells grown in culture (Dunlap, 1984b). How is it possible to constrain growth yet maintain maximal light emission?

There are several factors that have been explored in lab cultures: nutrient, oxygen and iron limitation, and the effects of osmolarity.

1. Nutrient limitation

In well aerated cultures the synthesis of luciferase and other components of the luminescent system is maximum in mid to late log phase: the specific activity of luminescence peaks at late log, and in some species is much ($\sim 100\times$) greater in cells grown in a rich medium than in those grown in a minimal medium (Table 2; Nealson et al., 1970). By itself, this last fact suggests that controls over growth and the synthesis of the luminescent system can be independent, and that energy and/or nutrient limitation *per se* will not favor luminescence over growth. However, in *Vibrio harveyi*, cells grow slowly and are weakly luminescent in a minimal medium: the addition of arginine greatly stimulates luminescence without affecting growth (Table 2), a possible model for the light organ. Mutants that are bright in a minimal medium

Table 2. Levels of luminescence *in vivo* and *in vitro* (extractable luciferase) obtained during growth of *Vibrio harveyi* on complex, minimal, and arginine-supplemented minimal media.[a]

Media	Luminescence intensities (quanta per sec per ml)	Cell density optical density at 600 nm	Viable count cells per ml	Quanta per sec per cell	Quanta per sec per optical density unit
Complex					
In vivo	4×10^{12}	12.5	5×10^{9}	8×10^{2}	3×10^{11}
In vitro	4×10^{12}	12.5	5×10^{9}	8×10^{2}	3×10^{11}
Minimal					
In vivo	4×10^{7}	0.15	4×10^{8}	0.01	2.7×10^{8}
In vitro	5×10^{8}	0.15	4×10^{8}	0.1	3.3×10^{9}
Minimal + arginine					
In vivo	1.2×10^{10}	0.4	10^{9}	12	3×10^{10}
In vitro	2×10^{10}	0.4	10^{9}	20	5×10^{10}

[a] From Nealson et al., 1970. Measurements were made in each experiment at the time when the luminescence per cell was at a maximum. In minimal medium, for example, luminescence peaked at a low cell density and the cells were smaller (compare cell density with the viable cell count). In the last two columns, it is shown that, based on cell mass, the luciferase content of cells grown in minimal is about 5% of those grown in complex, whereas those grown in minimal plus arginine contain almost twice as much as the cells in complex.

have been isolated and characterized (Fig. 2), and luminescence in such mutants is no longer stimulated by arginine (Waters and Hastings, 1977). This indicates that a high level of synthesis and expression of luciferase can occur in cells whose growth is severely limited.

At low cell densities in both rich and minimal media, growth occurs but there is no synthesis of the luminescent system. This has been attributed to the production and accumulation in the medium of a substance termed autoinducer, which controls transcription of the luminescent system (Nealson et al., 1970; Nealson, 1977). Indeed, autoinducer from *Vibrio fischeri* has been identified and synthesized (Eberhard et al., 1981); other species appear to utilize analogous but non-cross reactive (structurally different) autoinducers (Greenberg et al., 1979).

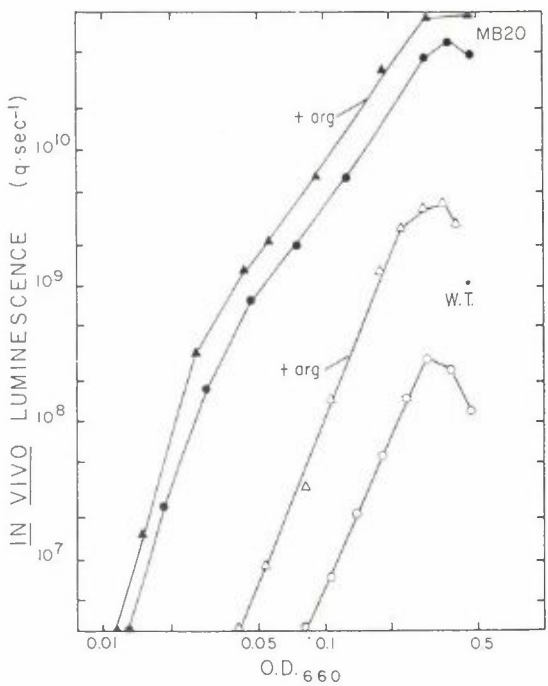

Figure 2. Effect of arginine on the development of *in vivo* luminescence during growth in minimal medium of both wild type (open symbols) and a "minimal bright" (MB) mutant, MB-20 (solid symbols). Cells from late-exponential-phase cultures were diluted 200-fold into fresh minimal medium. Arginine (final concentration, 3×10^{-3}M) was added at this time. Cell densities (abscissa) and *in vivo* luminescence (ordinate) of the cultures were measured with a side-arm flask. Symbols: circles, no addition; triangles, added arginine. Wild type in a complex medium (not shown) parallels the MB mutant (solid circles) from Waters and Hastings (1977).

The behavior of cultures subjected to dialysis (Fig. 3; Ulitzur and Hastings, 1979) and chemostat experiments (Fig. 4, Rosson and Nealson, 1981) have independently confirmed the autoinduction hypothesis. In the chemostat experiments, the addition of autoinducer to a carbon-limited slow-growing culture (growth rate, 0.04 h−1) resulted in the stimulation of luminescence by 10,000-fold, but it was still 10-fold less per cell than that of a less-limited but more rapidly growing culture (Fig. 4). It has been suggested that the confinement of the bacteria within the saccules or tubules allows autoinducer

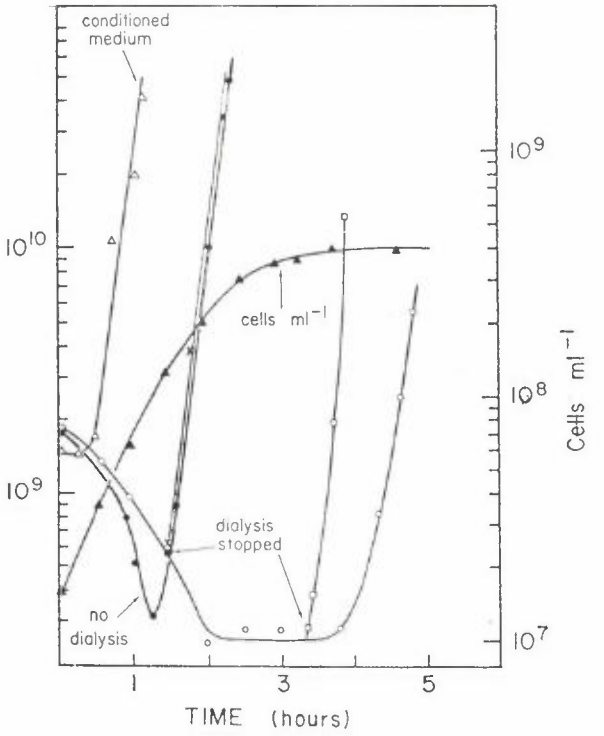

Figure 3. Luminescence of a culture grown with vigorous dialysis against fresh complex medium at 25°C. *Vibrio harveyi* wild-type cells were inoculated into complex medium in a hollow fiber dialysis unit (Bio-Rad Corp., BioFiber 50, 5,000-dalton cutoff, working surface 10,000 cm^2, dialyzed against fresh medium circulated at a rate of 20 ml/min during the first hour and at a rate of 200 ml/min thereafter). Samples were removed at the times indicated for measurement of luminescence (open circles) and cell density (solid triangles; Klett units). Dialysis was stopped in two such samples (arrows); autoinduction occurred promptly. In the culture kept in the hollow fiber unit, autoinduction occurred after about 4 hr in spite of continued dialysis, evidently due to the production of inducer at a rate faster than it could be removed. Two similar cultures were grown in flasks without dialysis, one in normal medium (solid circles) and one in a "conditioned" medium containing added autoinducer (open triangles) (after Ulitzur and Hastings, 1979).

to accumulate within the organ (Nealson, 1979; Dunlap, 1984b) but measurements of autoinducer levels in the organ (or elsewhere in the host, such as blood) have not been reported. That the luminescence systems of *V. harveyi* and *V. fischeri* are subject to catabolite repression and require cyclic AMP for induction (Nealson et al., 1972; Dunlap and Greenberg, 1985) suggest that its function involves nutrient limiting conditions.

Experiments that model the *in vivo* organ conditions better — high density, low growth rate — remain to be carried out. In the light organs of *Monocentris japonicus*, the doubling time (growth rate) of the symbiont has been estimated by the release of symbionts to be between 7.5 and 135 hr (0.005 to 0.09 hr^{-1}) (Haygood et al., 1984). By measuring the frequency of dividing cells, Dunlap (1984b) estimated the *in vivo* growth rate to be 0.03 hr^{-1} for *Photobacterium leiognathi* in the light organs of *Gazza minuta*; the same method can be used to convert 2% division forms in the monocentrid light organs (Tebo et al., 1979) to a growth rate of 0.013 hr^{-1}. It appears then, that the growth rates in the organs are at least 20 times slower than in laboratory culture experiments, yet the bacteria in the organs may reach densities of 6.4×10^9 to 1.0×10^{11} bacteria/ml in leiognathid light organs (Hastings and Mitchell, 1971; Dunlap, 1984b).

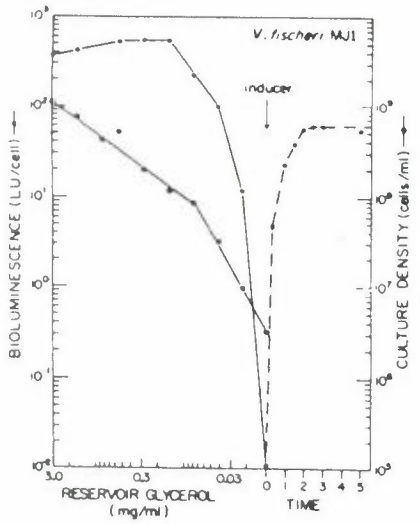

Figure 4. Changes in *Vibrio fischeri* bioluminescence (upper curve) and culture density at steady state as a function of glycerol concentration at a dilution rate of D = 0.4 hr^{-1}. At low glycerol, where luminescence was below the measurable level, purified autoinducer (250μl; 20μg/ml) was added (arrow) and the development of bioluminescence was measured as a function of time in hours. No increase in cell density occurred (after Rosson and Nealson, 1981).

2. Oxygen limitation

It has long been known that bioluminescence in bacteria can continue unabated at very low oxygen concentrations, where aerobic growth may be severely restricted (Harvey, 1952; Hastings, 1952, Lloyd et al., 1985). More recently it has been shown that in some strains of *P. phosphoreum* and *V. fischeri*, both known to be symbionts, luciferase synthesis continues briskly while growth is limited, resulting in cells with a much higher specific luciferase content than those grown in air (Fig. 5, right panel; Nealson and Hastings, 1977). This is a feature of only some species and strains; in

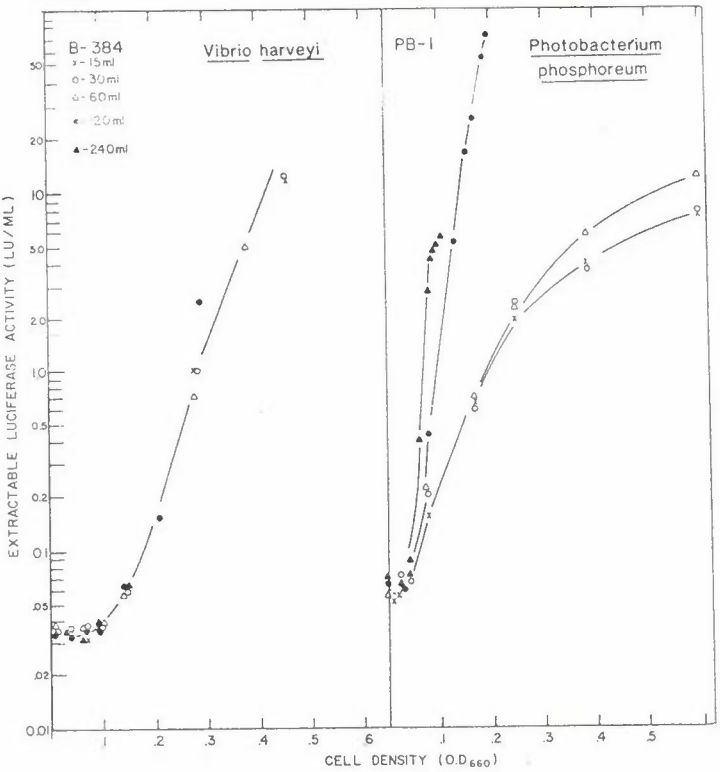

Figure 5. *Vibrio harveyi* and *Photobacterium phosphoreum* grown in a series of flasks containing different culture volumes from 15 ml to 240 ml, with gentle shaking, such that the dissolved oxygen concentration was progressively lower in flasks containing greater volumes. In *P. phosphoreum* both the *in vivo* luminescence and the extractable luciferase per cell were greater in cultures grown at lower oxygen, whereas in *Vibrio harveyi*, the restriction of cell density by low oxygen results in similar and proportional restrictions on luminescence (after Nealson and Hastings, 1977).

V. harveyi, which is not known to occur as a light-organ symbiont, luminescence *in vivo* and *in vitro* luciferase activity are both strictly related to cell density, and independent of the oxygen concentration in the medium during cell growth (Fig. 5, left panel). The fact that in both cases luminescence and luciferase per cell are much greater at higher cell densities is attributed to autoinduction.

In leiognathid fishes, where the light organ abuts the swim bladder, McFall-Ngai (1981; 1983) has shown that the oxygen concentration in the gas bladder can affect the intensity of luminescence. She suggests that the gas bladder, rather than blood circulation, is the major source of oxygen for the organ, and that the bacteria in the organ could be oxygen-limited (Dunlap and McFall-Ngai, 1987). As it happens, luciferase synthesis in *Photobacterium leiognathi* was not stimulated by low oxygen (Nealson and Hastings, 1977). A role for oxygen in the regulation of growth and luminescence in this species should nevertheless not be ruled out.

In no case is it known what carbon and energy are actually provided for the bacteria by the host. Ruby and Nealson (1976, 1977) found that the monocentrid symbiont, *V. fischeri*, grown aerobically in pure culture with glucose, excreted large amounts of pyruvate, which could then be metabolized by the bacteria when the glucose was depleted. The authors suggested that, at low oxygen in the light organ the pyruvate might be utilized instead by the unusual mitochondria of the epithelial cells lining the tubules containing the bacteria. In this model (Nealson, 1979), the mitochondrial pyruvate oxidation would serve to maintain a low O_2 concentration within the tubules.

3. Iron limitation

Similar observations concerning growth rate and luciferase synthesis have been made with regard to low iron concentrations, in both *V. harveyi* and *V. fischeri* (Makemson and Hastings, 1982; Haygood and Nealson, 1984, 1985), as well as *P. leiognathi* (Dunlap, 1984a). The rates of growth and oxygen consumption of cells in a medium containing low iron is decreased, but the synthesis of luciferase and the other components of the luminescent systems are enhanced (Fig. 6). It is estimated that about 20% of the oxygen consumption is attributable to the luciferase pathway (Makemson, 1986; Dunlap, 1984b); this value is probably greater in cells growing in low iron.

Makemson and Hastings (1982) stressed the implications of low iron conditions in *V. harveyi* for the possible function of luciferase as a terminal oxidase alternate to cytochrome. Under these conditions the cellular level

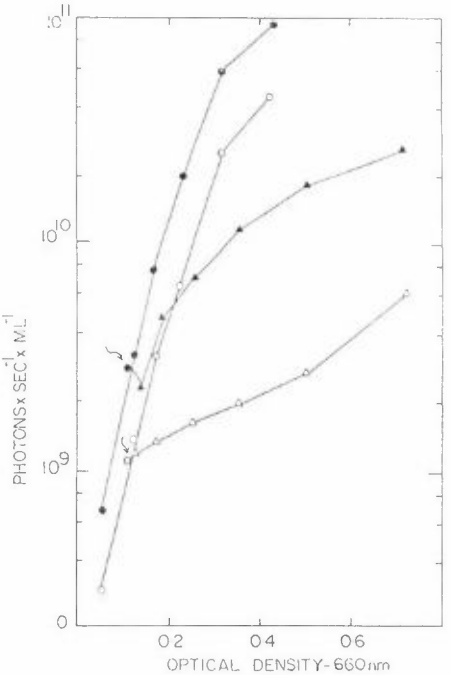

Figure 6. Iron repression of *in vivo* bioluminescence (open symbols) and the synthesis of luciferase (solid symbols) in *Vibrio harveyi* growing in HEPES-glutamate minimal medium without added iron (circles). The cultures were split and iron added at the arrows (triangles; 4.3μM ferric ammonium citrate) (after Makemson and Hastings, 1982).

of cytochromes are decreased while luciferase synthesis is enhanced, and as a terminal oxidase luciferase appears to support growth (Makemson, 1986; Makemson and Hastings, 1986). Further, added iron can repress luciferase synthesis and alter the response to catabolite repression, suggesting that outside the symbiotic situation the regulation of the luciferase system may be very different.

Haygood and Nealson (1984, 1985) emphasized the ecological implications of low iron for light organ symbionts. Iron limitation, in contrast to carbon, nitrogen or phosphorus limitation, inhibits growth but not luminescence in *V. fischeri*, possibly due to a decrease in cytochromes. Fish possess serum transferrins active in sequestering iron (Bobak et al., 1984; Stratil et al., 1983, 1985), so that in the light organs iron could be withheld from the symbionts. Indirect evidence that this may be the case is that the luminous symbiont *P. leiognathi* contains a copper/zinc superoxide dismutase

(bacteriocuprein) in addition to the traditional iron and manganese superoxide dismutase (Dunlap and Steinman, 1986 and references therein). This suggests that the symbionts would be able to resist oxygen toxicity in the absence of available iron through the activity of their bacteriocupreins.

4. Osmotic control

Early studies on luminous bacteria revealed that the optimal salt concentrations for growth and luminescence may differ. Farghaly (1950) observed optimum growth of "*Achromobacter fischeri*" at 1.5% NaCl while its luminescence peaked at 3% NaCl. He considered these effects to be osmotic in nature, as clearly shown in the recent work of Dunlap (1985).

Differential effects of different salt concentrations on growth and luminescence have since been reported for many species and strains by many workers (for example see Henry and Michelson, 1970; Kossler, 1970; Makiguchi et al., 1980; Watanabe and Hastings, 1986). But the results appeared to be conflicting, since in some cases quite the opposite effect — optimal growth at 3% NaCl but maximum luminescence at 1% NaCl — was found. Some of the earlier studies were compromised by the lack of an adequate bacterial taxonomy; the matter was resolved by Dunlap (1984a), who examined several different species in a single study and reported that there are indeed distinctly different patterns (Fig. 7). In *P. leiognathi* the optimum salt concentration for growth is near that of sea water while the maximum luminescence, the intensity of which corresponds to that of cells taken directly from leiognathid light organs, occurs at a lower concentration, equal to about 30% sea water. In *P. phosphoreum* on the other hand, the situation is essentially reversed: luminescence is optimal above 3% NaCl while growth is favored in a lower osmolarity. In a third symbiotic species, *V. fischeri*, the pattern is intermediate in that growth and luminescence are similarly affected by osmolarity.

Dunlap (1984a) thus proposed that the physiological differences between bacterial species with regard to osmolarity, as determined with pure cultures in the lab, may correlated with the osmotic conditions maintained in the organs where the particular species occur, such that restricted growth and maximum luminescence are favored. In leigonathid fishes, which have internal light organs harboring *P. leiognathi*, it was proposed that osmolarity is low; marine fishes do osmoregulate their body fluids and tissues at 30 to 40% sea water (Fange et al., 1976; Griffith, 1981). In contrast, macrourids, morids and trachichthyids, which harbor *P. phosphoreum*, bear light organs leading

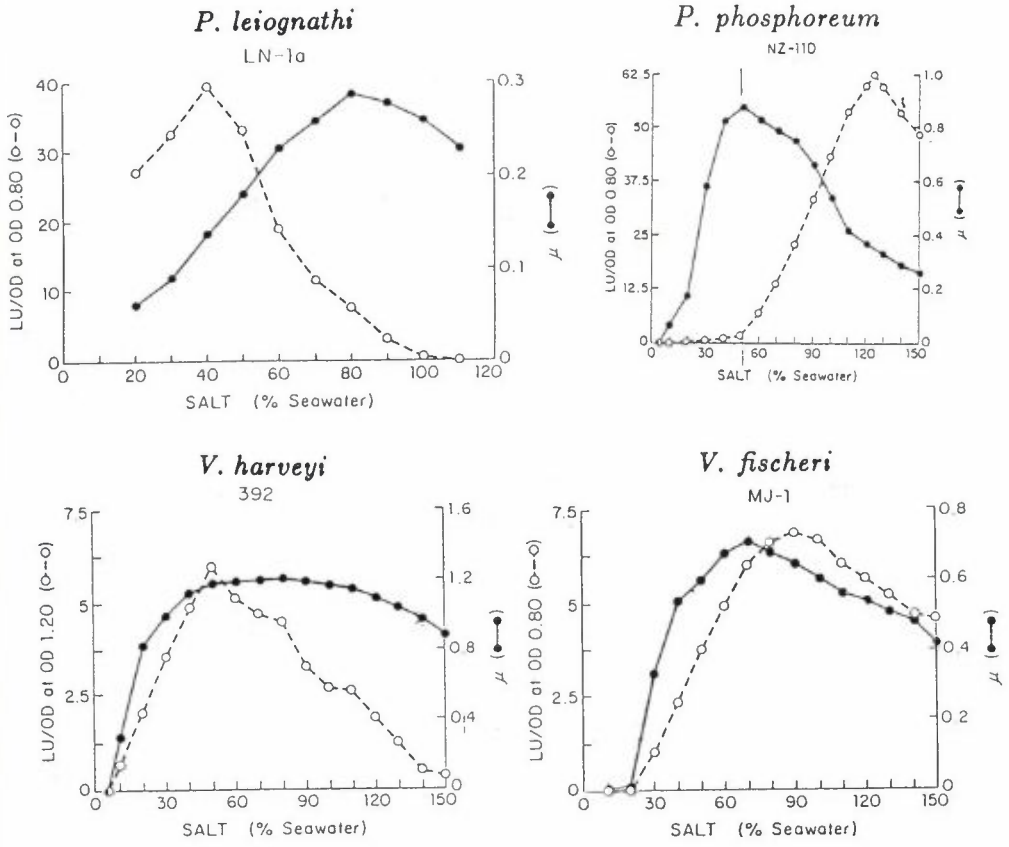

Figure 7. Effect of salt concentration upon growth and *in vivo* luminescence in 4 species of luminous bacteria. *P. leiognathi* was grown in a basal minimal medium (C source, glycerol) with different amounts of sea water salts (expressed as percentage of sea water, where 30 gm/l = 100%); all other species were grown in a basal complete medium (with yeast extract and peptone) with varying amounts of sea water salts (after Dunlap, 1984a).

off the hindgut (Herring and Morin, 1978). Since marine fish eliminate some salts via the gut (Prosser, 1973; Gordon, 1977), it may be that the light organs are involved and thus provide a high salt (equal to or greater than sea water) environment. For monocentrid fishes, which harbor *V. fischeri*, the light organ is located externally on the lower jaw (Tebo et al., 1979), communicating directly with sea water. As a consequence, the light organ might have an osmolarity somewhat higher than internal tissues. Compared to *P. leiognathi* and *P. phosphoreum*, which exhibit optimal luminescence at low and high osmolarity respectively, the luminescence of *V. fischeri* symbiont strains is optimal in lab cultures at an intermediate osmolarity, only slightly lower than sea water itself. Since both growth rate and luminescence are optimal at similar salt concentrations, other control factors are likely to be more important for *V. fischeri*.

3. Acquisition of Symbiotic Bacteria

All of the light organs being considered (Fig. 1) have openings to the outside, either directly to the sea water or via the alimentary canal (Herring and Morin, 1978; Kessel, 1977; Tebo et al., 1979). It is possible, even probable, that a fish host acquires its bacterial symbionts through these openings. At the same time the luminous organs themselves, with an overflow from bacterial growth, are a likely origin of the inocula. Fish with openings directly to the outside (monocentrids and anomalopids) have been shown to release luminous bacteria into the sea water (Haygood et al., 1984; Nealson et al., 1984).

Planktonic luminous bacteria come not only from light organs; fecal contents of mid-water fishes and invertebrates are extremely rich sources (Raymond and DeVries, 1976; Robison and Morin, 1977;Baross et al., 1978; Andrews et al., 1984; Dilmore and Hood, 1986). In the deep sea fishes, the psychrotrophic *P. phosphoreum* is almost always the light organ symbiont (Ruby and Morin, 1978). The greatest proportion of the benthic and coastal luminous fishes utilize symbiotic bacteria as their source of light compared to luminous oceanic fishes which can have their own, nonbacterial luciferase/luciferin systems.

From whatever source, luminous bacteria are ubiquitous in sea water. The counts of viable planktonic luminous bacteria (colony forming units, including all species) range up to 10^4 per liter of sea water (O'Brien and Sizemore, 1979; Ruby et al., 1980; Ruby and Nealson, 1978; Ruby and Morin, 1979; Singleton and Skerman, 1973; Yetinson and Shilo, 1979). The distribution of different species of luminous bacteria in the water column down to 10,000 m was measured by Ruby et al. (1980). There occurred a population of luminous bacteria between 250 and 1000 m which was comprised almost entirely of *P. phosphoreum*. This distribution (at depth) remained relatively stable on three visits to the same station over 6 months. A different species (*V. harveyi*) was predominant in the surface water, down to 50 m, and its abundance was seasonally variable, as found in studies of coastal locations (Ruby and Nealson, 1978; Yetinson and Shilo, 1979).

The ability of luminous bacteria to survive in sea water varies, depending upon nutrient, temperature, light and other factors (Shilo and Yetinson, 1979). Some distributions, such as that of *P. leiognathi*, are directly attributable to photodestruction (from sunlight). Yetinson and Shilo (1979) showed that the seasonal appearance of *V. fischeri* and winter and summer forms of *V. harveyi* and winter and summer forms of *V. harveyi* in the eastern

Mediterranean is correlated with temperature. A similar seasonally related species change was reported in the coastal waters off San Diego by Ruby and Nealson (1978). Symbiont bacteria do decline in numbers in aquaria after the host fish has been removed (Haygood et al., 1984; Nealson et al., 1984).

Some investigators have found that luminous bacteria, whether symbionts or not, can survive for long periods in sea water when suspended in sea water at low densities ($< 10^5$ bacteria/ml). *V. harveyi*, *V. fischeri* and *P. phosphoreum* may remain viable under such conditions for longer than 4 years without forming dark mutants (Eberhard, 1972 and private communication).

In sum, sea water appears to be an abundant reservoir of luminous bacteria that would ensure the probability of infection for developing light organs in communication with sea water or digestive tracts. Establishment of the bacterial association appears to occur very early in the life cycle of fish, but there is little information as to exactly when (Yamada et al., 1979; Dunlap and McFall-Ngai, 1987). Leis and Bullock (1986) recently studied developing light organs in larvae of the apogonid fish, *Siphamia versicolor* (which harbors *P. leiognathi*). In a larva 2.8 mm in length, no bacteria were observed in the developing light organ, but bacteria were present in larvae 3.5 mm in length.

4. Light Organ Symbiont Specificity: How is the Symbiont Species Selected and a Pure Culture Maintained?

Studies with different species in culture have established at least three means for growth restriction without limitation of luminescence: control of oxygen, iron and osmolarity. Nutrient control may also be involved. While some earlier models have tacitly assumed that only one of these factors would be operative in a given fish light organ, growth and luminescence of bacterial symbionts may actually be subject to control by a multiplicity of factors: how they are poised quantitatively in a particular light organ could be involved in determining the bacterial species that becomes dominant.

Indeed, to the extent that the conditions responsible for physiological control of growth in a light organ are idiosyncratic to a particular species or strain, then host control of such factors could favor a particular organism. The paradox, of course, is that restriction of growth is the key to mutualism, yet potential (non-luminescent) contaminants must be subject to an even more stringent growth restriction if they are to be excluded. How can this be achieved? One possibility is that the luminescent system itself may provide a partial escape from growth limitation, as has been suggested to occur under conditions of low oxygen and/or low iron (Makemson and Hastings,

1986), and low osmolarity (Dunlap, 1984b; 1985). Thus, while the growth of all bacteria is very restricted under these conditions, luciferase respiration (Makemson, 1986) might provide a modest advantage for luminous organisms, such that under "chemostat" conditions in the light organ, the luminous strains are selected. Osmolarity, then, might serve as the selective condition for a particular luminous species.

To be sure, other factors may be involved. For example, fish lectins could bind receptors on the symbiont cell surface, thereby enhancing nutrient exchange, or fish IgA type antibodies and complement (or other lysins) could react with all but the symbionts (for which the host fish are immunologically tolerant). Inhibitory substances (e.g. antibiotics) to which the symbionts are resistant could be produced by the host or the bacteria themselves. In fact, strains of *P. leiognathi* isolated as symbionts do elaborate substances inhibitory for growth of other bacteria (Hastings and Nealson, 1972), and potent antibiotics are known to be produced by terrestrial luminous bacterial symbionts (Paul et al., 1981). Such substances would help assure the maintenance of a pure culture once the inoculation of the light organ with the right symbiont occurred.

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